Bioorganic & Medicinal Chemistry Letters 20 (2010) 5207–5211

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Total synthesis and biological evaluation of tambjamine K and a library of unnatural analogs

Leslie N. Aldrich [a], Sydney L. Stoops [b], Brenda C. Crews [c], Lawrence J. Marnett [a,b,c,d], Craig W. Lindsley [a,b,d,*]

[a] Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA
[b] Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[c] Department of Biochemistry, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[d] Vanderbilt Institute of Chemical Biology, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 14 June 2010
Revised 29 June 2010
Accepted 30 June 2010
Available online 23 July 2010

Keywords:
Tambjamine
Cancer
Total synthesis

ABSTRACT

Herein we disclose the first total synthesis of tambjamine K and a library of unnatural analogs. Unnatural analogs were shown to be more potent in viability, proliferation, and invasion assays than the natural product in multiple cancer cell lines, with minimal to no cytotoxicity on non-transformed cell lines.

The tambjamines A–J (**1**–**10**) are a 2,2′-bipyrrolic class of cytotoxic alkaloids with diverse aliphatic termini isolated from bacteria and marine invertebrates and related to the tripyrolic prodigiosin **11** family (Fig. 1).[1–8] Members of this class have demonstrated a wide range of biological activities including antitumor, antimicrobial, and immunosuppressive properties. For tambjamines D (**4**) and E (**5**), the antitumor properties have been correlated with DNA intercalation and oxidative cleavage of single-strand DNA.[9] In a recent Letter in this journal, Gavagnin and co-workers described the isolation and characterization of a new member of the tambjamine family, tambjamine K (**13**), isolated from the Azorean nudibranch *Tambja ceutae*.[10] Like other members of this family, **12** displayed antiproliferative and cytotoxicity against tumor (CaCo-2, IC_{50} = 3.5 nM, HeLa IC_{50} = 14.6 μM, C6 IC_{50} = 14 μM, H9c2 IC_{50} = 2.7 μM, and 3T3-L1 IC_{50} = 19 μM) and non-tumor cell lines. Interestingly, **12** displayed differential effects across these tumor cell lines with a variance of >5000-fold (CaCo-2 vs 3T3-L1).[10] Based on these data and our own efforts in related areas, we initiated an effort for the total synthesis and biological evaluation of **12** along with a library of unnatural analogs with unprecedented diversity in the eastern C7 position to survey moieties other than aliphatic alkyl chains.

Interest in the tambjamine family originated in our evaluation[11] of Fenical's biosynthetic proposal[12] for synthesis of marineosin A (**14**) via an intramolecular inverse-electron demand Diels–Alder reaction with prodigiosin analog **13** (Fig. 2). Like **1**–**12**, marineosin A displayed potent cytotoxic activity against HCT116 cells (IC_{50} = 500 nM).[11,12]

Our synthetic approach to access **12** was similar to that we employed for the synthesis of **13**.[11] As shown in Scheme 1, a Vilsmeier–Haack haloformylation was performed on 4-methoxy-3-pyrrolin-2-one **15** to provide bromoenamine **16** in 59% yield. Suzuki coupling with Boc-1*H*-pyrrol-2yl boronic acid **17** delivered the Boc protected analog **18** in 48% yield. Finally, an acid mediated condensation between **18** and isopentyl amine **18** afforded tambjamine K (**12**) in 65% yield and 18% overall yield for the three step sequence. Synthetic **12** was identical in all aspects to the natural product.[13]

While **12** was studied in a number of tumor cell lines, it was not evaluated in cell viability assays with HCT116 colorectal carcinoma or MB231 breast carcinoma cell lines—tumor lines of interest to our lab.[14] Moreover, we had not yet evaluated **13** or another related prodigiosin analog **19** we employed as a template for an intermolecular inverse-electron demand Diels–Alder (IEDDA) reaction to access the marineosin A core.[11] As shown in Table 1, tambjamine K (**12**) displayed weak cytotoxicity against HCT116 (IC_{50} = 13.7 μM) and MB231 (IC_{50} = 15.3 μM). In contrast, the intramolecular IEDDA prodigiosin analog **13** was more potent with IC_{50} values of 3.5 μM for both tumor lines. The intermolecular IEDDA prodigiosin analog **19** was found to be extremely potent, with IC_{50} values of 146 nM and 362 nM, for HCT116 and MB231 cell lines, respectively.

These data prompted us to synthesize and evaluate a library of unnatural tambjamine analogs[15] to capitalize on the SAR observed for this class of natural products akin to our earlier work developing unnatural analogs with activities beyond the natural product.[16–18]

* Corresponding author. Tel.: +1 615 322 8700; fax: +1 615 343 6532.
E-mail address: craig.lindsley@vanderbilt.edu (C.W. Lindsley).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.154

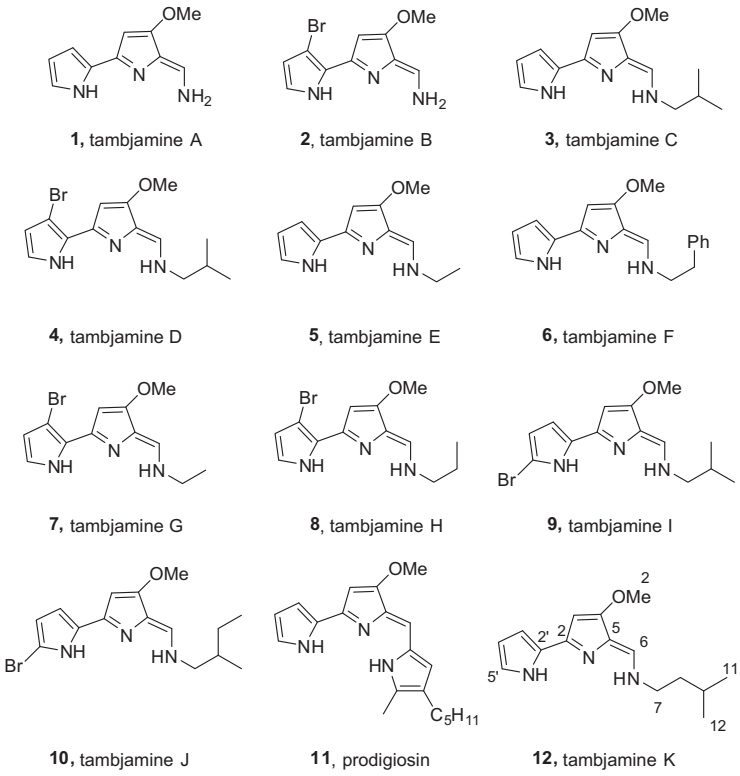


Figure 1. Structures of the tambjamines A–J (**1**–**10**), prodigiosin (**11**) and the newly discovered tambjamine K (**12**).

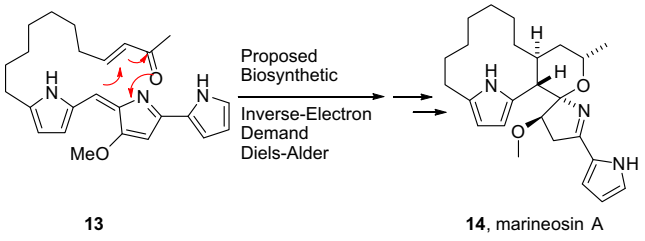
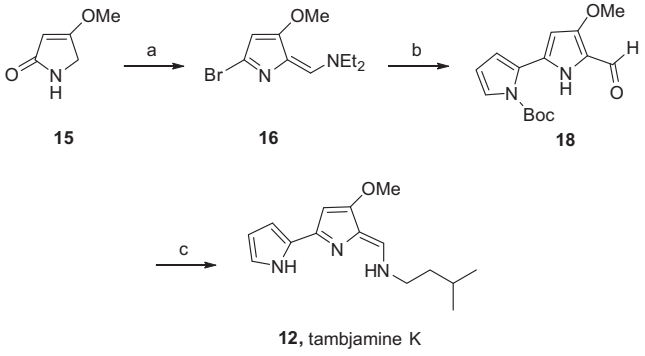


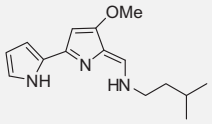
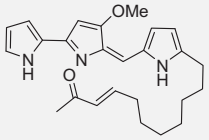
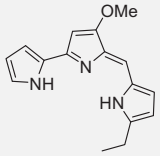
Figure 2. Proposed biosynthesis of marineosin A (**14**) via an inverse-electron demand Diels–Alder reaction with prodigiosin analog **13**.



Scheme 1. Reagents and conditions: (a) (i) $POBr_3$, $HCONEt_2$, CH_2Cl_2, 0–40 °C, 3.5 h; (ii) 15% NaOH until pH ~7, 59%; (b) (i) $Pd(OAc)_2$, PPh_3, toluene, 70 °C, 20 min; (ii) Boc-1*H*-pyrrol-2yl boronic acid (**17**), 9:1 dioxane/H_2O, Na_2CO_3, 85 °C, 3 h, 48%; (c) isopentyl amine, 0.87 M HCl, MeOH, rt, 6 h, 65%.

Importantly, Quinn and co-workers[19] previously prepared a combinatorial library of 10 unnatural tambjamine analogs, but all possessed limited diversity with respect to R^2 and aliphatic side chains dominated.

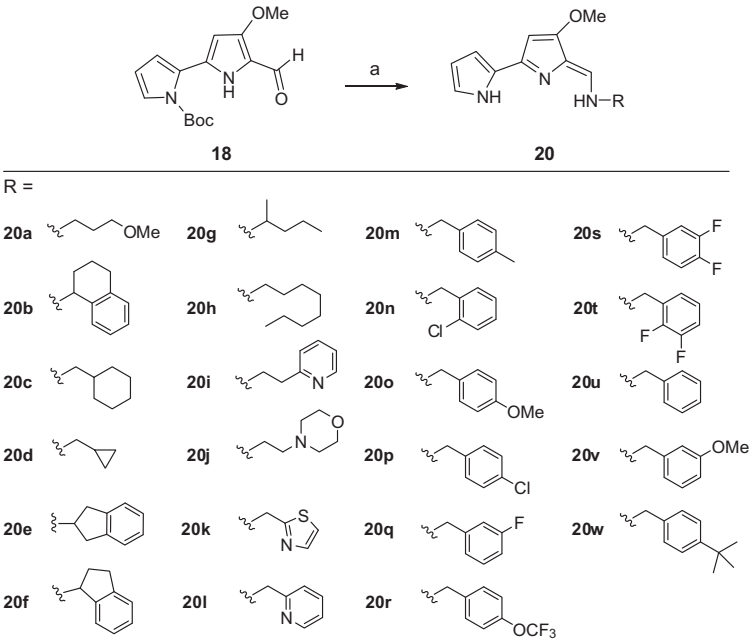
Table 1
Structures and activities of tambjamine K and unnatural analogs

Compd	Structure	HCT116 IC_{50}[a] (μM)	MBA231 IC_{50}[a] (μM)
12		13.7	15.3
13		3.6	3.5
14		0.14	0.36

[a] 8000 cells/well in 96-well plate followed by 24 h for attachment. Added vehicle or compounds in RPMI 1640 plus 10% FBS + penicillin–streptomycin. Cells allowed to grow for 48 h, then viability was assessed.

Our library was designed to incorporate functionalized benzyl, heteroaryl moieties and other previously undescribed analogs with varying degrees of lipophilicity and basicity to further develop SAR. The library was prepared as shown in Scheme 2, and differed from the route to access **12** only in extended reaction time, as several amines proved sluggish in their conversion to unnatural tambjamine analogs **20**; however, all analogs were successfully prepared in yields ranging from 35% to 88%.[15]

We triaged the library of analogs **20** by a employing a 10 μM single point screen in the standard 48 h viability assay using both HCT116 and MB231 cells.[14] The majority of analogs, especially the



Scheme 2. Reagents and conditions: RNH_2, 0.87 M HCl, MeOH, rt to 50 °C, 24–48 h, 35–88%.

benzyl congeners, **20m**–**20w**, had no effect on viability in either tumor cell line. Figure 3 shows the single point data for the analogs **20** with activity in these assays, and it is important to note that differential activity was noted between the two tumor cell lines. The most potent analog was **20b**, a racemic 1,2,3,4-tetrahydronaphthalene congener, which significantly decreased HCT116 viability (<10% viability) while having only marginal effect on MB231 (40% viability). Other unnatural analogs **20c**, **20e**, **20f**, and **20g** decreased HCT116 cell viability to less than 20%, with minimal effect on MB231 viability. Clearly, constraining the benzyl amine moiety in **20b**, **20e**, and **20f** into a bicyclic ring system is important for activity relative to the inactive benzyl derivatives **20m**–**20w**. Based on the promising single point data, we then determined IC_{50}s for **20b** in both cell lines. **20b** displayed moderately potent cytotoxicity against HCT116 cells (IC_{50} = 1.8 μM) with the concentration–response curve (CRC) reaching baseline (0% viability at 10 μM dose). In contrast, **20b** displayed weak cytotoxicity in the MB231 cells (IC_{50} = 4.0 μM) with the CRC only achieving ~50% decrease in cell viability at the highest (10 μM) dose; therefore, the calculated IC_{50} is for a partial response. Moreover, in our standard cytotoxicity assay in non-transformed cells, **20b** displayed no toxicity.

Based on these data, we then evaluated select unnatural analogs with activity in the HCT116 cell viability assay and evaluated them in standard 48 h cell proliferation assays (Fig. 4) using another colorectal line (SW620) and a non-small cell lung carcinoma (NSCLC) line (H520).[20] Interestingly, **19**, the most potent tambjamine analog in both the HCT116 (IC_{50} = 146 nM) and MB231 (IC_{50} = 362 nM) viability assays, had no effect on proliferation in either the SW620 or the H520 cell lines. However, unnatural analog **20b**, displayed a significant effect on inhibiting proliferation in both tumor cell lines, while other analogs showed varying effects.

As both unnatural tambjamine analogs **19** and **20e** displayed minimal to no effect on proliferation (viability) in SW620 cells, we examined their ability to block invasion in this tumor line, as the ability of tumor cells to invade into the surrounding microenvironment is the defining step in tumor progression.[21] As shown in Figure 5, both **19** and **20e** significantly blocked invasion, with **19** completely inhibiting invasion. Moreover, both analogs displayed

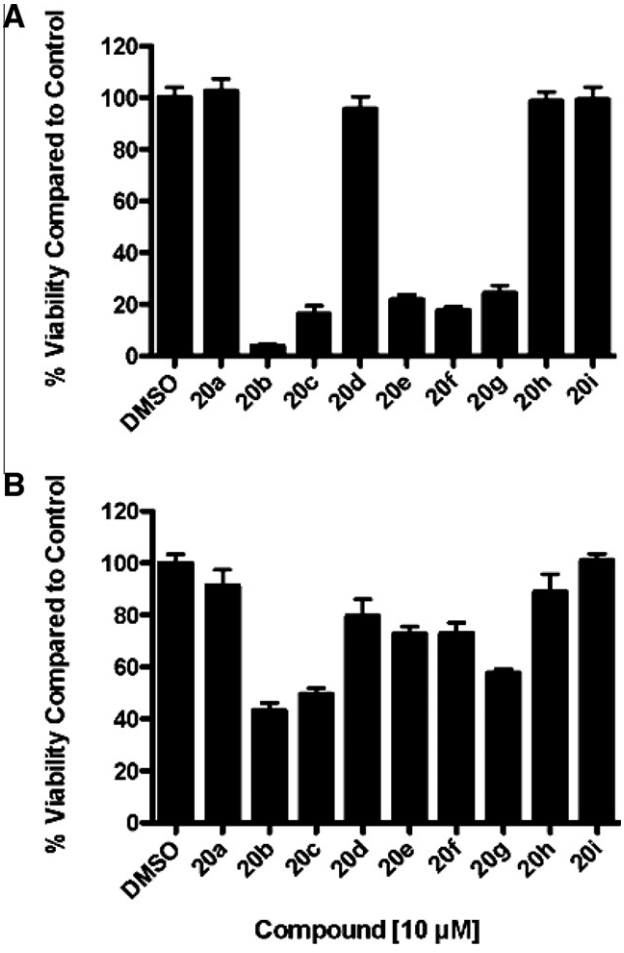


Figure 3. Single point (10 μM) screen of library of analogs **20**. (A) 48 h cell viability assay with HCT116 colorectal carcinoma line; (B) 48 h cell viability assay with MDA MB231 breast carcinoma line.

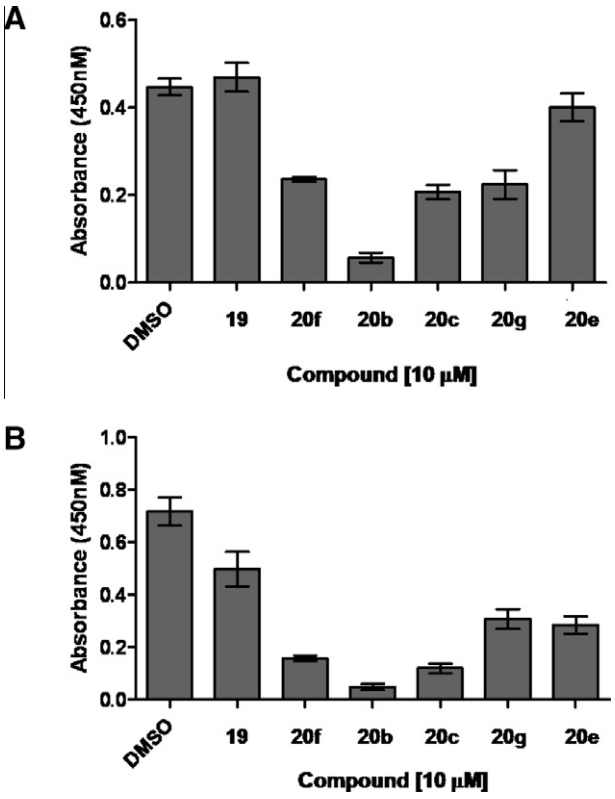


Figure 4. Single point (10 μM) screen of select unnatural tambjamine analogs in 48 h cell proliferation assays. (A) Proliferation assay with SW620 colorectal carcinoma line; (B) proliferation assay with H520 NSCLC line.

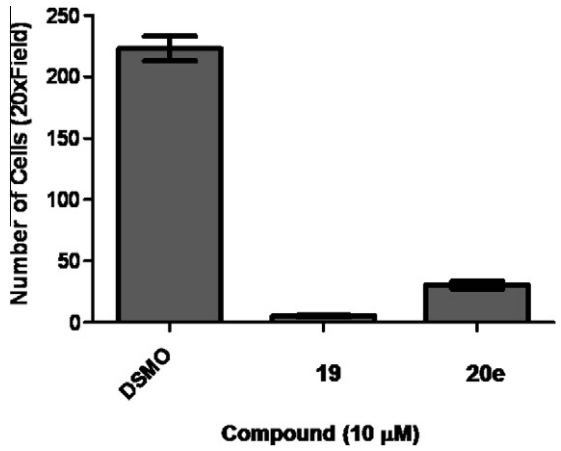


Figure 5. Single point (10 μM) screen of select unnatural tambjamine analogs in 24 h cell invasion assay in the SW620 colorectal carcinoma line.

minimal or no cytotoxicity in this colorectal tumor cell line, further highlighting the value of unnatural analog synthesis.

In summary, we completed the first total synthesis of tambjamine K (**12**) in 18% overall yield coupled with evaluation in viability assays in both colon (HCT116) and breast cancer (MB231) cell lines. We also prepared a library of unnatural tambjamine analogs with unprecedented diversity and improved biological activity against a number of tumor cell lines in viability, proliferation and invasion assays. This effort demonstrated that subtle changes to the tambjamine core afford varying degrees of selectivity against different tumor cell lines. These data argue for further exploration of the tambjamine scaffold coupled with evaluation (viability, proliferation, and invasion) in additional human tumor cell lines. Current efforts are focused on synthesizing a second generation library including the discrete enantiomers of **20b** and **20e**, chiral α-methyl congers of the benzylic analogs **20m**–**20w** and more focused analogs based on **19**. In parallel, we are working to identify the molecular target(s) for these unnatural analogs by evaluating **19**, **20b**, and **20e** against large panels of kinases, growth factor and phosphatases as a primary approach. These efforts are in progress and will be reported in due course.

Acknowledgments

The authors thank the Department of Pharmacology and the Vanderbilt Institute of Chemical Biology (VICB) for support of this research. L.N.A. acknowledges a predoctoral fellowship from the VICB, and S.L.S. acknowledges a support from the Pharmacology Predoctoral Training Grant (NIH 5T326M007628-30).

References and notes

1. Carte, B.; Faulkner, D. J. *J. Org. Chem.* **1983**, *48*, 2314.
2. Lindquist, N.; Fenical, W. *Experientia* **1991**, *47*, 504.
3. Kazlauskas, R.; Marwood, J. F.; Murphy, P. T.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 215.
4. Wrede, F.; Hettche, O. *Ber. Dtsch. Chem. Ges. B.* **1929**, *62*, 2678.
5. Wasserman, H. H.; Friedland, D. J.; Morrison, D. A. *Tetrahedron Lett.* **1968**, *24*, 641.
6. Carte, B.; Faulkner, D. J. *J. Chem. Ecol.* **1986**, *12*, 795.
7. Pinkerton, D. M.; Banwell, M. G.; Willis, A. C. *Org. Lett.* **2007**, *9*, 5127.
8. Blackman, A. J.; Li, C. *Aust. J. Chem.* **1994**, *47*, 1625.
9. Melvin, M. S.; Calcutt, M. W.; Noftle, R. E.; Manderville, R. A. *Chem. Res. Toxicol.* **2002**, *15*, 742.
10. Carbone, M.; Irace, C.; Costagliola, F.; Villani, G.; Calado, G.; Padula, V.; Cimino, G.; Cervera, J. L.; Santamaria, R.; Gavagnin, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2668.
11. Aldrich, L. N.; Dawson, E. S.; Lindsley, C. W. *Org. Lett.* **2010**, *12*, 1048.
12. Boonlarppraadab, C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2008**, *10*, 5505.
13. *Experimental for the total synthesis of tambjamine K* (**12**): *(E)-N-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-N-ethylethanamine* (**16**): A 100 mL round bottom flask was charged with *N*,*N*-diethylformamide (2.86 g, 28.29 mmol) and 23 mL of dichloromethane. The mixture was cooled to 0 °C, and a solution of phosphorous oxybromide (10.14 g, 35.36 mmol) in dichloromethane (7 mL) was added slowly over 20 min. After addition was complete, the reaction mixture was stirred for an additional 20 min. A solution of 4-methoxy-3-pyrrolin-2-one (**15**) (2.00 g, 17.68 mmol) in dichloromethane (18 mL) was added dropwise over 10 min, and the mixture was stirred for an additional 20 min. The flask was then removed from the ice bath, transferred to an oil bath, and refluxed (42 °C) for 3.5 h. The reaction mixture was then transferred to a 500 mL round bottom flask, cooled to 0 °C, and quenched by dropwise addition of water (20 mL). Sodium hydroxide (3.0 M in H_2O, 230 mL) was slowly added and the mixture was stirred for an additional 20 minutes. The layers were separated and the aqueous layer was extracted with dichloromethane (3×). The combined organic layers were dried over sodium sulfate and concentrated in vacuo. The resulting yellow oil was subjected to flash chromatography (silica, 4:1 hex/EtOAc) to give **16** as a tan solid (2.67 g 59%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.29 (t, J = 7.2 Hz, 3H), 1.30 (t, J = 7.2 Hz, 3H), 3.40 (q, J = 7.2 Hz, 2H), 3.76 (s, 3H), 4.13 (q, J = 7.2 Hz, 2H), 5.59 (s, 1H), 6.99 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 12.4, 14.5, 44.5, 51.0, 57.9, 96.4, 120.7, 133.6, 138.5, 165.3. IR (KBr) ν_{max} 2975, 2934, 1629, 1529, 1408, 1290, 1264, 1195, 1116, 1072, 906, 737 cm^{-1}. HRMS: $C_{10}H_{16}N_2OBr$. Calcd: [M+H], 259.0446, found: [M+H], 259.0448.
tert-Butyl 5′-formyl-4′-methoxy-1H,1′H-2,2′-bipyrrole-1-carboxylate (**18**): $Pd(PPh_3)_4$ was generated in situ by adding triphenylphosphine (1.22 g, 4.64 mmol) to a magnetically stirred suspension of palladium II acetate (0.23 g, 1.03 mmol) in degassed toluene (5.0 mL) then heating the ensuing mixture at 70 °C for 20 min under an atmosphere of argon. A solution of *N*-Boc-pyrrole-2-boronic acid **17** (3.26 g, 15.46 mmol) and bromoenamine **16** (2.67 g, 10.31 mmol) in H_2O/1,4-dioxane (1:9 v/v, 86 mL) was degassed, purged with argon gas and added to the solution of $Pd(PPh_3)_4$ in toluene. Anhydrous sodium carbonate (3.28 g, 30.93 mmol) was added and the reaction mixture stirred at 85 °C. After 3.5 h, the mixture was cooled and poured into water (150 mL). The solution was reduced to pH 7 using 2 M HCl, partitioned with dichloromethane, and extracted (4×). The organic layers were combined, dried over sodium sulfate, and condensed in vacuo to give a brown residue that was purified by flash chromatography (silica, 4:1 hex/EtOAc) affording aldehyde **18** as an orange solid (1.44 g, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.61 (s, 9H), 3.88 (s, 3H), 6.07 (d, J = 3.5 Hz, 1H), 6.24 (t, J = 3.5 Hz, 1H) 6.66 (dd, J = 3.5 Hz, 1.76 Hz, 1H), 7.33 (dd, J = 3.4, 1.80 Hz, 1H), 9.53 (s, 1H), 10.73 (br s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 27.8, 57.8, 85.7, 94.7, 111.4, 116.8, 118.2, 124.5, 125.9, 130.2,

149.6, 157.6, 174.3. IR (KBr) ν_{max} 3221, 2979, 2833, 1735, 1623, 1549, 1502, 1433, 1370, 1330, 1287, 1255, 1140, 1021 cm^{-1}. HRMS: $C_{15}H_{19}N_2O_4$. Calcd: [M+H] 291.1345, found: [M+H] 291.1345.

Tambjamine K (**12**): isopentylamine (36.0 mg, 0.414 mmol) was added to a stirred suspension of aldehyde **18** (100 mg, 0.345 mmol) in methanol (5.0 mL) at room temperature, followed by 0.87 M HCl in methanol (0.6 mL). After 6 h, the reaction was quenched with saturated sodium bicarbonate, extracted with dichloromethane (3×), and washed with brine (1×). The organic layers were combined, dried over anhydrous sodium sulfate, and concentrated in vacuo to give a dark brown residue. This residue was purified by reverse phase chromatography using acetonitrile and 0.1% TFA/water (gradient: 15:85–55:45) to give tambjamine K (**12**) as an orange oil that solidified upon standing (58 mg, 65%). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (d, J = 6.8 Hz, 6H), 1.62 (q, J = 6.8 Hz, 2H), 1.71 (m, 1H), 3.48 (q, J = 6.8 Hz, 2H), 3.93 (s, 3H), 5.98 (d, J = 2.4 Hz, 1H), 6.28 (m, 1H), 6.75 (m, 1H), 7.09 (m, 1H), 7.33, (d, J = 15.2 Hz), 9.93 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 22.1, 25.2, 38.7, 49.2, 58.3, 91.5, 110.5, 110.6, 113.6, 122.2, 124.5, 140.0, 143.2, 164.0. IR (KBr) ν_{max} 3234, 2960, 2917, 1674, 1605, 1529, 1463, 1428, 1368, 1203, 1137, 968, 728, 721 cm^{-1}. HRMS: $C_{15}H_{22}N_3O$. Calcd: [M+H], 260.1763, found: [M+H], 260.1764.

14. Daniels, R. N.; Melancon, B. J.; Wang, E. A.; Crews, B. S.; Marnett, L. M.; Sulikowski, G. A.; Lindsley, C. W. *J. Org. Chem.* **2009**, *74*, 8852.
15. *General method for the synthesis of tambjamine unnatural analogs* (**20**): primary amine (0.517 mmol) was added to a stirred suspension of aldehyde **16** (30 mg, 0.103 mmol) in methanol (2.0 mL) at room temperature. 0.87 M HCl in methanol (0.6 mL) was subsequently added, and the reaction was stirred for 24 h. Reactions that had not reached completion by this time were stirred at 50 °C for an additional 24-h period. Reaction mixtures were concentrated, and crude compounds were purified by reverse phase chromatography using acetonitrile and 0.1% TFA/water to give tambjamine unnatural analogs **20** in yields ranging from 35–88%.
16. Kennedy, J. P.; Brogan, J. T.; Lindsley, C. W. *J. Nat. Prod.* **2008**, *71*, 1783.
17. Kennedy, J. P.; Conn, P. J.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3304.
18. Lewis, J. A.; Daniels, N. R.; Lindsley, C. W. *Org. Lett.* **2008**, *10*, 4545.
19. Davis, R. A.; Carroll, A. R.; Quinn, R. J. *Aust. J. Chem.* **2001**, *54*, 355.
20. *Viability assay method*: SW620 and H520 cells (2.5×10^4/100 μl) were seeded in 96-well microtiter plates prior to treatment. Cells were treated with 10 μM concentration of synthesized compound in quadruplicate for 24 h and 48 h in RPMI 1640 Supplemented media and 100 μg/ml penicillin–streptomycin. The Quick Cell Proliferation Assay Kit from BioVision (Mountain View, CA) was used to measure proliferation. The RPMI media is removed and replaced with 100 μl of the WST-1/ECS reagent diluted 1:10 in RPMI Supplemented media. The plates are incubated for 1 h at 37 °C in 5% CO_2 in the air. The change in proliferation is quantified by measuring the absorbance of the dye solution at 450 nm on a microtiter plate reader.
21. *Invasion assay method*: SW620 cells (1.0×10^6/ml) were seeded in 6 mm round dish prior to treatment. Cells were treated with 10 μM concentration of synthesized compound for 24 h in RPMI 1640 supplemented media and 100 μg/ml penicillin–streptomycin. 40 μl (2.5 mg/ml) of BD Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA) was added to top of insert of 24-well transwell permeable support plates with polycarbonate membrane (Corning Inc, Corning, NY). Then the cells were trypsinized and 3×10^5/250 μl cells were added to the top of the chamber in serum free RPMI media, and 1 ml of RPMI media with 10% FBS was added to the bottom of the well. Then the plates were incubated for 72 h at 37 °C in 5% CO_2 in the air. Then the wells were stained with 1% crystal violet in 50% methanol for 1 h and washed in PBS. The membrane was cut off, adhered to a slide with glycerol, and analyzed in 20× field via microscopy. 3–20× fields were quantified per membrane.